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Long-term reduction of plasma homocysteine levels by super-flux dialyzers in hemodialysis patients

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Background. Hyperhomocysteinemia is an independent risk factor for cardiovascular disease in chronic hemodialysis (CHD) patients. Treatment with folic acid normalizes total homocysteine (tHcy) in only a minority of the patients. The present investigation has been conducted to study the influence of various dialyzers with different flux characteristics on the reduction of tHcy in the long term.

Methods. Total Hcy, folate, vitamin B_6, vitamin B_{12}, and albumin levels were assessed prospectively in 10 patients undergoing HD with high-flux polysulfon (PS; F 60) and 20 patients with super-flux dialyzers ($N = 10$ PS, F 500S; $N = 10$ CTA, Tricea 150G). Blood samples were collected before hemodialysis both at the beginning of the study and after 12 weeks.

Results. At baseline, all the groups showed similar tHcy levels. During high-flux dialysis, tHcy remained stable. In contrast, during dialysis with both super-flux modalities, tHcy decreased significantly (F 500S week 1, 29.6 ± 9.9 μmol/L, and week 12, 21.5 ± 8.5 μmol/L, $P = 0.007$; Tricea 150G week 1, 24.4 ± 8.7 μmol/L, and week 12, 15.3 ± 3.7 μmol/L, $P = 0.008$). The difference between high-flux and super-flux dialyzers was highly significant (mean: high-flux increase 15.6%, super-flux decrease 33.3%, $P = 0.001$). Multivariate analysis showed a significant effect of super-flux dialysis on tHcy ($P = 0.001$), independently of the previously mentioned variables.

Conclusions. Our findings clearly show that both types of super-flux dialyzers reduced tHcy significantly. As the molecular weight of free homocysteine is less than 268 D, the most likely explanation seems to be the removal of uremic toxins with inhibitory activities against enzymes involved in the extrarenal homocysteine metabolism.

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in chronic hemodialysis (CHD) patients. The high prevalence of atherosclerotic disease has been related to increased rates of known risk factors, such as hypertension, hyperlipidemia, diabetes mellitus, and smoking. However, recent studies suggest that these risk factors do not fully explain the high prevalence of CVD in this patient group [1]. Recently, hyperhomocysteinemia has been established as an independent risk factor for CVD, not only in the general population [2–5], but also in CHD patients [6, 7]. As hyperhomocysteinemia occurs in approximately 85 to 100% of the dialysis population, it has been suggested that apart from the previously mentioned classic risk factors, elevated plasma homocysteine levels contribute substantially to the high incidence of CVD in these patients [14].

Homocysteine is a sulfhydryl amino acid, resulting from demethylation of the essential amino acid methionine. Homocysteine can be remethylated to methionine or transsulfurated to cysteine. There are two different remethylation pathways. The first reaction requires vitamin B_{12} as a cofactor and 5-methyltetrahydrofolate (5-MTHF), a folic acid metabolite, as methyldonor. The second reaction requires betaine as methyldonor. The transsulfuration pathway consists of two irreversible, vitamin B_6-dependent reactions (Fig. 1).

The pathophysiological mechanism of hyperhomocysteinemia in CHD patients is unclear. A lack of elimination as a result of a decreased renal clearance seems unlikely, as the urinary excretion of homocysteine is negligible in healthy subjects [8]. Accumulation caused by a decreased intrarenal metabolism of homocysteine in end-stage renal disease (ESRD) patients seems implausible as well. In contrast to experiments in rats [9], recent data in healthy volunteers showed no arteriovenous homocysteine differences in the kidneys, indicating that renal extraction does not occur in fasting humans [10]. Therefore, the inhibition by uremic toxins of enzymes involved in the extrarenal homocysteine removal has been suggested as an alternative explanation for the elevated homocysteine levels in ESRD patients [10, 11]. Indeed, according to a recent study, most of the abnor-

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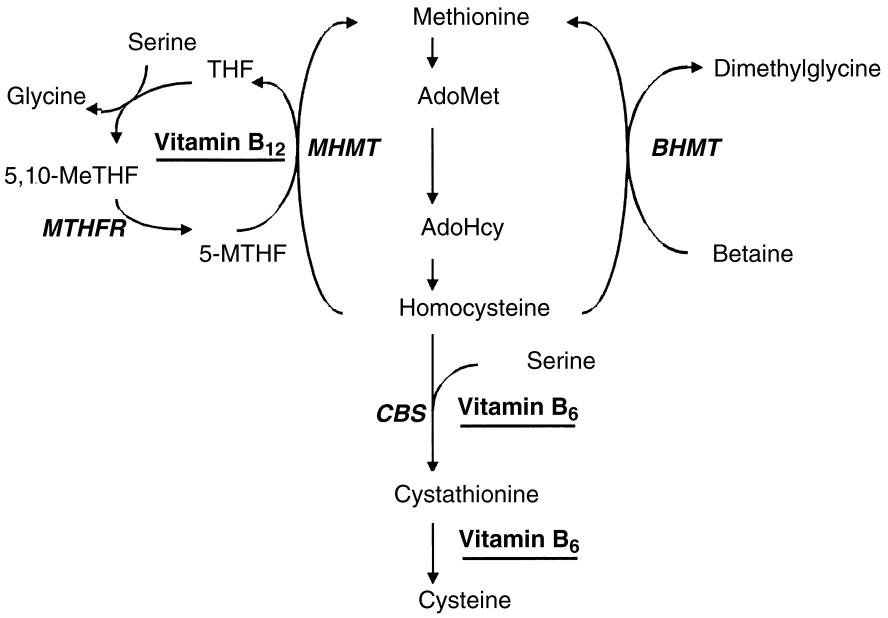

Fig. 1. Homocysteine metabolism. Abbreviations are: AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; THF, tetrahydrofolate; 5-MTHF, 5-methyltetrahydrofolate; 5,10-MeTHF, 5,10-methylene-tetrahydrofolate; *MTHFR*, 5,10-methylene-tetrahydrofolate reductase; *MHMT*, methyltetrahydrofolate-homocysteine methyltransferase; *BHMT*, betaine-homocysteine methyltransferase; *CBS*, cystathionine β-synthase.

malities of homocysteine metabolism in renal failure can be explained by a refractoriness of the folate and/or vitamin B_{12}-dependent remethylation pathway as mentioned previously in this article (Fig. 1) [12]. Several studies in CHD patients have shown that treatment with folic acid, either alone or in combination with other B vitamins, reduced total homocysteine (tHcy) levels markedly [13, 14]. However, with the exception of one recent report [15], vitamin supplementation failed to normalize plasma tHcy entirely in the vast majority of these patients [16, 17]. The effect of hemodialysis itself on plasma tHcy concentration has been investigated in only a small number of uncontrolled studies [18–21]. Interestingly, the reduction in tHcy was not markedly different between high- and low-flux devices [20]. If one accepts the view that hemodialysis can lower plasma tHcy only by elimination through the filter, the latter observation would be expected, given the molecular weight of free homocysteine in plasma (that is, 268 D or lower). However, using low-flux dialyzers, Arnadottir et al have shown that the creatinine concentration rose immediately after HD, whereas the reduction in plasma tHcy persisted for 8 to 20 hours [11]. Thereafter, a gradual increase was observed, reaching predialysis values after 44 hours. Based on these findings, it was suggested that the intradialytical decline resulted from the removal of unbound homocysteine. The reduction that persisted for 20 hours after HD was attributed to the elimination of uremic toxins with inhibitory activity against relevant enzymes, involved in the metabolism of homocysteine.

Therefore, it is conceivable that dialyzers with superior flux and/or adsorptive characteristics are capable of removing such toxins more effectively than conventional high-flux devices. Use of such dialyzers may thus lead to a more sustained reduction or even normalization of plasma tHcy in the long term. In this report, we describe the first prospective randomized and controlled study that examines the effect of three types of dialyzers with different flux characteristics (high- and super-flux) and membrane materials [polysulfon (PS) and cellulosic triacetate (CTA)] on plasma tHcy levels in CHD patients.

Table 1. Patient characteristics

	High-flux $N = 10$	Super-flux $N = 20$
Age *years*		
Median *range*	65 (25–86)	70 (21–82)
Gender		
Men	7	11
Women	3	9
Time on hemodialysis *months*		
Median *range*	39 (8–200)	30 (10–301)
Dialysis dose (Kt/V_{urea})	0.87	1.13
Residual creatinine clearance *mL/min*	0.44	0.87

METHODS

Patients

Thirty stable CHD patients (18 men and 12 women) with a median age of 69 years (range of 25 to 82) participated in the study after giving written informed consent. All patients had been on maintenance HD for at least eight months (median 32.5, range 8 to 301). Patient characteristics are shown in Table 1. Causes of renal insufficiency were hypertension ($N = 10$), diabetic nephropathy ($N = 4$), polycystic kidney disease ($N = 3$), focal

Table 2. Membrane characteristics

	F 60	F 500S	Tricea 150G
Inner lumen *μm*	200	155	200
Wall thickness *μm*	40	35	15
Ultrafiltration coefficient *mL/mm Hg/h*	40	300 (H_2O)	29
Surface area m^2	1.3	1.2	1.5
Clearance *mL/min*			
β_2-microglobulin (molecular weight 11.8 kD)	38	65–80	Not known
Sieving coefficient β_2-microglobulin	0.65	0.9	0.8

glomerulosclerosis ($N = 3$), chronic pyelonephritis ($N = 1$), chronic glomerulonephritis ($N = 3$), renal failure of unknown origin ($N = 4$), and others ($N = 2$). Exclusion criteria were hemodialysis for less than eight months, vitamin B_{12} supplementation before entering the study, severe malnutrition (albumin <25 g/L), and current use of anticonvulsant drugs. All patients received folic acid, 5 mg twice a week, 1 mg vitamin B_6 daily, whereas neither betaine nor vitamin B_{12} was prescribed to the participants of the study. Approval of the local ethical committee was obtained.

Study design

Before participating in the study, all patients were dialyzed three times a week using a high-flux PS dialyzer. After computerized randomization, the patients were dialyzed either with a high-flux PS, a super-flux PS, or a super-flux CTA dialyzer for 12 weeks. Blood samples were collected both at the start of the study and after 12 weeks. Blood samples were drawn from the afferent line before dialysis and analyzed for tHcy, vitamin B_6, vitamin B_{12}, folate, and albumin.

Dialysis procedure and materials

The dialysis sessions lasted three to five hours, depending on the individual prescription of the patient. Only first-use dialyzers were used. Characteristics of the three dialyzers used in this study (PS, F 60 and F 500 S; Fresenius, Bad Homburg, Germany; CTA, Tricea 150 G; Baxter, Osaka, Japan) are depicted in Table 2. According to the individual needs of the patients, blood flow and ultrafiltration (UF) rates were kept constant between 200 and 250 mL/min and 300 to 1000 mL/hours, respectively. Isolated UF was not performed. Bicarbonate dialysate was used with a dialysate flow of 500 mL/min. Anticoagulation was achieved by dalteparin with an initial dose of 2500 to 6000 IU, followed by an extra dose of 500 to 1000 IU during the dialysis treatment if necessary. Individual conditions (blood flow, UF, dalteparin dose) were kept stable throughout the study period.

Analytical methods

After collection, the blood samples were immediately centrifuged and stored at −20°C until required for testing.

Homocysteine. Total homocysteine was measured in NaFK2-oxalate plasma by means of high-performance liquid chromatography (HPLC) with fluorescence detection according to the method of Ubbink, Vermaak, and Bissbort [22]. The upper limit of the reference range was <15 μmol/L. The intra-assay coefficient of variation was about 8%.

Folate and vitamin B_{12}. Serum levels were determined by using an automated luminiscence-immuno-assay (LIA, ACS: 180; Bayer, Mijdrecht, The Netherlands). Reference ranges were >5 nmol/L and 150 to 670 pmol/L, respectively, and the intra-assay coefficient of variation ranged from 5 to 10%.

Vitamin B_6. Vitamin B_6 (pyridoxal 5′-phosphate) was established in heparin blood, applying HPLC with post-column derivation (Analytico, Center for Research, Breda, The Netherlands). The reference range was 40 to 100 nmol/L, and the intra-assay coefficient of variation ranged from 5 to 8%.

Albumin. Serum concentrations were measured using nephelometry (Behring Nephelometer II; Behring, Leusden, The Netherlands). The reference interval ranged from 35 to 52 g/L. The intra-assay coefficient of variation was approximately 5%.

Adequacy of dialysis. Kt/V_{urea} was measured using an urea monitor (Biostat; Baxter, Utrecht, The Netherlands). This measurement was performed by means of a membrane bound urease and an ammonium-ion–selective electrode, which measures the ammonium generated by the reaction: urea $\rightarrow_{(urease)}$ammonia (upper and lower detection levels of urea, 1.0 to 28.5 mmol/L and precision 1.8%). Correction for the two-compartment model was performed by the formula of Daugirdas: $eKt/V = Kt/V\,[1 - (0.06/T)] + 0.03$ [23].

Statistical analysis

Data are expressed as mean ± SD or median and range when appropriate. Analysis was performed with the Statistical Package for Social Sciences/PC+ software system using paired and unpaired *t* tests to study the differences between groups. Correlation coefficients were calculated with the Pearson test. Multivariate analysis was performed to test the relationship between tHcy and different dialysis modalities. Differences were considered statistically significant at $P < 0.05$.

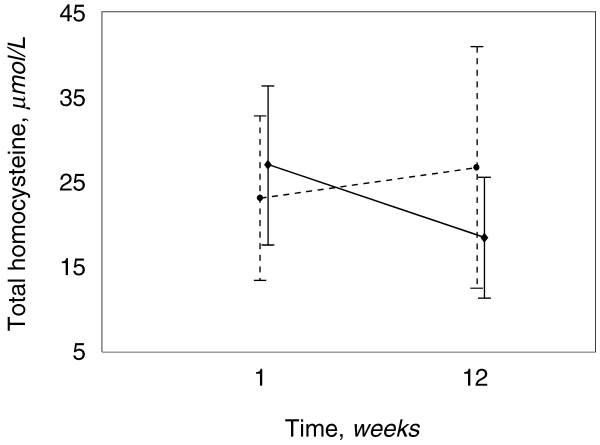

Fig. 2. Plasma concentrations of total homocysteine (tHcy) in μmol/L (mean ± SD) before hemodialysis at the start of the study and after 12 weeks with a high-flux dialyzer (●; PS, F 60) and both super-flux devices (◆; PS, F 500S; CTA, Tricea 150G). During dialysis with both super-flux devices, tHcy levels decreased significantly (*P = 0.001).

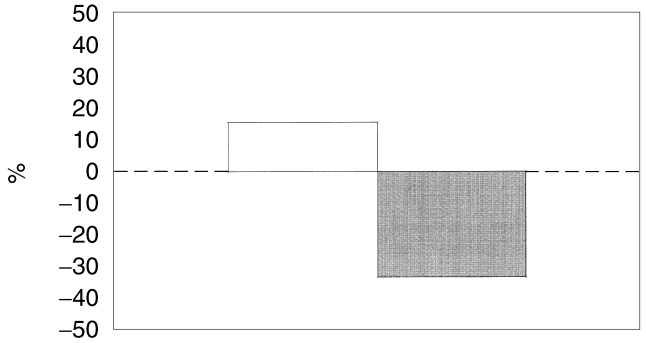

Fig. 3. Clearance of tHcy in percentage of high-flux (□; F 60) and super-flux (■; F 500S and Tricea 150G) dialysis. A significant difference was observed between these modalities during 12 weeks of treatment (*P = 0.001). No marked differences were observed between both super-flux dialyzers.

RESULTS

Vitamin B_6, vitamin B_{12}, folate, and albumin

At baseline, none of the patients had a deficiency of vitamin B_{12}. Two patients had subnormal vitamin B_6 levels, and one patient had a subnormal folate level, probably because of noncompliance. The mean serum folate level was 126.6 ± 103.6 nmol/L. Serum vitamin B_{12} was 354.4 ± 163.7 pmol/L. Serum vitamin B_6 was 185.6 ± 148.4 nmol/L, and serum albumin was 36.9 ± 3.9 g/L. No significant differences were observed between the three study groups.

Homocysteine

At baseline, all the groups showed comparable tHcy levels (F60, 23.1 ± 9.7 μmol/L; F500S, 29.6 ± 9.9 μmol/L; and Tricea 150G, 24.4 ± 8.7 μmol/L, P = 0.28).

High-flux. During high-flux dialysis, tHcy levels remained stable (week 1, 23.1 ± 9.7 μmol/L; week 12, 26.7 ± 14.2 μmol/L, P = 0.25). The percentage of patients with normal tHcy level did not change.

Super-flux. In contrast, during dialysis with both super-flux dialyzers, tHcy levels decreased significantly (F 500S, 29.6 ± 9.9 μmol/L in week 1 vs. 21.5 ± 8.5 μmol/L in week 12, P = 0.007; Tricea 150G, 24.4 ± 8.7 μmol/L in week 1 vs. 15.3 ± 3.7 μmol/L in week 12, P = 0.008). Marked differences were not found between the two super-flux dialyzers (F 500S, −8.1 ± 7.3 μmol/L; Tricea 150G, −9.1 ± 8.5, P = 0.78).

High-flux versus super-flux. The change in tHcy levels during the study period was significantly different between the high-flux device and the two super-flux dialyzers, either alone or combined [F 60 vs. F 500S: F 60 + 15.6%, F 500S −27.4%, P = 0.006; F 60 vs. Tricea 150G: F 60 + 15.6%, Tricea 150G −37.3%, P = 0.005; F 60 vs. (F 500S + Tricea 150G): F 60 + 15.6%, [F 500S + Tricea 150G] −33.3%, P = 0.001]. The combined data are shown in Figures 2 and 3. Both before and after three months of high-flux dialysis, normal values of tHcy (<15 μmol/L) were observed in only 20% of the patients, whereas super-flux dialysis showed normalization in 50% of the patients after three months.

Relationship between concentrations of vitamin cofactors, albumin, patient-related characteristics, and homocysteine

Both at the beginning of the study and after 12 weeks, no significant correlations were found between vitamin cofactors, albumin, age, sex, time on dialysis, residual creatinine clearance, Kt/V, or tHcy.

Multivariate analysis, comparing high-flux and super-flux dialysis, showed a significant effect of super-flux dialysis on tHcy levels [F = 13.58; degree of freedom (1, 27), P = 0.001]. No interaction was found with the previously mentioned factors, which are known to influence tHcy levels.

DISCUSSION

The present study was designed to evaluate three different dialyzers, including two types of super-flux devices, with respect to their long-term effects on tHcy levels in CHD patients. Interestingly, both super-flux dialyzers (F 500S and Tricea 150G) reduced plasma tHcy more effectively than the high-flux dialyzer (F 60) used in this study. After 12 weeks of super-flux dialysis, tHcy was within the reference range in 50% of the patients, in contrast to 20% after high-flux HD. This finding may have important consequences in clinical practice as hyperhomocysteinemia is common in CHD patients and associated with an adverse cardiovascular outcome [7, 24].

There is no unifying theory that explains the pathophysiological mechanism of hyperhomocysteinemia. Growing evidence indicates that hyperhomocysteinemia exerts its toxic effects by promoting oxidative stress. During auto-oxidation of homocysteine, various reactive oxygen species are formed [25]. In vitro studies have shown that hyperhomocysteinemia is associated with impaired endothelium-dependent relaxation, which is attributed to a reduced bioavailability of nitric oxide [26, 27]. So far, these data are consistent with the idea that hyperhomocysteinemia contributes to the development of atherosclerosis. Alternatively, however, it has been postulated that hyperhomocysteinemia results from cell injury rather than being the cause of it [28]. Whatever the underlying mechanism and pathophysiology of hyperhomocysteinemia in ESRD patients, long-term normalization of plasma tHcy seems desirable for three reasons. First, low homocysteine levels are clinically associated with a low cardiovascular risk profile [7]. Second, hyperhomocysteinemia is associated with cytotoxic effects on various cell types, such as thrombocytes, leukocytes, and endothelial cells [abstract; Guo et al, *Neth J Med* 52 (Suppl 1):S58, 1998] [29]. Third, a drop in plasma tHcy may alleviate the endothelial injury, provoked by the uremic state itself.

At first glance, our results seem to contradict a recent study comparing a low-flux PS device (F 8) with a high-flux PS dialyzer (F 80) [30]. After a period of three months, neither the F 8 nor the F 80 device showed any effect on plasma tHcy levels in CHD patients. However, in fact, these results are entirely in agreement with our observations with the high-flux F 60 dialyzer, which is almost identical to the F 80 as far as the physicochemical characteristics are concerned. Only the membrane surface area (F 80 1.8 m^2; F 60 1.3 m^2) and the UF factor (F 80 50 mL/mm Hg/hour; F 60 40 mL/mm Hg/hour) differ between these two dialyzers. As for super-flux HD, plasma tHcy was markedly reduced after three months of treatment, which seems to be a rational finding. Super-flux dialyzers have been designed to maximize convective transport by increasing the pressure drop along the fibers of the membrane (F 500S) [31, 32]. Furthermore, an increase in the pore size (F 500S) and/or an in homogeneous distribution of both pores and pore sizes (Tricea 150G; J. Vienken, personal communication) increase the permeability of the membrane. Consequently, these characteristics allow the transfer of large (protein bound) uremic toxins across the membrane, as indicated by an increased sieving coefficient for β_2-microglobulin (Table 2).

As mentioned previously in this article, accumulation of uremic toxins with inhibitory activities against enzymes involved in the extrarenal metabolism of homocysteine has been suggested as a possible cause for the state of hyperhomocysteinemia in CHD patients. Whether the tHcy reduction, as observed in our study after three months of HD with super-flux dialyzers, depends on the removal of such substances is currently under investigation.

It is of note that super-flux CTA and PS membranes showed a comparable decline after 12 weeks of treatment. Interestingly, as shown by multivariate analysis, the use of super-flux dialyzers was the only significant predictor in reducing plasma tHcy. In contrast to other investigations [33, 34], our study showed no clear correlations between folate, vitamin B_6, vitamin B_{12}, albumin concentration, and tHcy levels, either at the start or after 12 weeks. As for serum folate, the lack of correlation may be due to supraphysiological values resulting from abundant supplementation.

In conclusion, our findings clearly show that hemodialysis with super-flux dialyzers is more effective in reducing plasma tHcy levels than high-flux dialyzers. The decline was independent of other factors, which are known to influence plasma tHcy levels. Whether long-term normalization of tHcy levels results in improvement of endothelial dysfunction and a reduction of CVD in CHD patients deserves further study.

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REFERENCES

1. Excerpts from the USRDS 1996 Annual Data Report. *Am J Kidney Dis* 28(Suppl):S79–S102, 1996
2. Boushey CJ, Beresford SAA, Omen GS, *et al*: A quantitative assessment of plasma homocysteine as a risk factor for cardiovascular disease. *JAMA* 274:1049–1057, 1995
3. Arnesen E, Refsum HM, Bonaa KH, *et al*: Serum total homocysteine and coronary heart disease. *Int J Epidemiol* 24:704–709, 1995
4. Perry IJ, Refsum HM, Morris RW, *et al*: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 346:1395–1398, 1995
5. Graham IM, Daly LE, Refsum HM, *et al*: Plasma homocysteine as a risk factor for vascular disease. *JAMA* 277:1775–1781, 1997
6. Robinson K, Gupta A, Dennis V, *et al*: Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 94:2743–2748, 1996
7. Bostom AG, Shemin D, Verhoef P, *et al*: Elevated fasting total plasma homocysteine levels and cardiovascular disease outcomes in maintenance dialysis patients. *Arterioscler Thromb Vas Biol* 17:2554–2558, 1997
8. Refsum HM, Helland S, Ueland PM: Radioenzymic determination of homocysteine in plasma and urine. *Clin Chem* 31:624–628, 1985
9. Bostom AG, Brosnan JT, Hall B, *et al*: Net uptake of plasma homocysteine by the rat kidney in vivo. *Atherosclerosis* 116:59–62, 1995
10. Van Guldener C, Donker AJM, Jacobs C, *et al*: No net renal

extraction of homocysteine in fasting humans. *Kidney Int* 54:166–169, 1998
11. Arnadottir M, Berg AL, Hegbrant J, *et al*: Influence of hemodialysis on plasma total homocysteine concentration. *Nephrol Dial Transplant* 14:142–146, 1999
12. Henning BF, Riezler R, Tepel M, *et al*: Evidence of altered homocysteine metabolism in chronic renal failure. *Nephron* 83: 314–322, 1999
13. Janssen MJFM, van den Berg M, Stehouwer CDA, *et al*: Hyperhomocysteinemia: A role in the accelerated atherogenesis of chronic renal failure. *Neth J Med* 46:244–251, 1995
14. Van Guldener C, Janssen MJFM, Lambert J, *et al*: No change in impaired endothelial dysfunction after long-term folic acid therapy of hyperhomocysteinemia in hemodialysis patients. *Nephrol Dial Transplant* 13:106–112, 1998
15. Touam M, Zingraff J, Jungers P, *et al*: Effective correction of hyperhomocysteinemia in hemodialysis patients by intravenous folinic acid and pyridoxine therapy. *Kidney Int* 56:2292–2296, 1999
16. Janssen MJFM, van Guldener C, de Jong GMT, *et al*: Folic acid treatment of hyperhomocysteinemia in dialysis patients. *Miner Electrolyte Metab* 22:110–114, 1996
17. Van Guldener C, Janssen MJFM, de Meer K, *et al*: Effect of folic acid and betaine on fasting and postmethionine-loading plasma homocysteine and methionine levels in chronic hemodialysis patients. *J Intern Med* 245:175–183, 1999
18. Hultber B, Andersson A, Sterner G: Plasma homocysteine in renal failure. *Clin Nephrol* 40:230–234, 1993
19. Tamura T, Johnston KE, Bergman SM: Homocysteine and folate concentrations in blood from patients treated with hemodialysis. *J Am Soc Nephrol* 7:2414–2418, 1996
20. Biasioli S, Schiavon R, Petrosino L, *et al*: Dialysis kinetics of homocysteine and reactive oxygen species. *ASAIO J* 44:423–432, 1998
21. Suliman ME, Anderstam B, Linhol B, *et al*: Total, free and protein bound sulphur amino acids in uraemic patients. *Nephrol Dial Transplant* 12:2332–2338, 1997
22. Ubbink JB, Vermaak WJH, Bissbort S: Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. *J Chromatogr* 265:441–446, 1991
23. Daugirdas JT: *Chronic Hemodialysis Prescription*: *Handbook of Dialysis.* Boston, Little, Brown, 1994
24. Moustapha A, Naso A, Nahlawi M, *et al*: Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 97:138–141, 1998
25. Jones BG, Rose FA, Tudball N: Lipid peroxidation and homocysteine induced toxicity. *Atherosclerosis* 105:165–170, 1994
26. Stamler JS, Osborne JA, Jaraki O, *et al*: Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and relate oxides of nitrogen. *J Clin Invest* 91:308–318, 1993
27. Chow K, Cheung F, Lao TTH, *et al*: Effect of homocysteine on the production of nitric oxide in endothelial cells. *Clin Exp Pharmacol Physiol* 26:817–818, 1999
28. Dudman NPB: An alternative view of homocysteine. *Lancet* 354:2072–2074, 1999
29. Dudman NPD, Temple SE, Guo XW, *et al*: Homocysteine enhances neutrophil-endothelial interactions in both cultured human cells and rats in vivo. *Circ Res* 84:409–416, 1999
30. House AA, Wells GA, Donnelly JG, *et al*: Randomized trial of high-flux vs. low-flux haemodialysis: Effects on homocysteine and lipids. *Nephrol Dial Transplant* 15:1029–1034, 2000
31. Dellanna F, Wuepper A, Baldamus CA: Internal filtration: Advantage in haemodialysis? *Nephrol Dial Transplant* 11:83–86, 1996
32. Ronco C: Backfiltration in clinical dialysis: Nature of the phenomenon, mechanisms and possible solutions. *Int J Artif Organs* 13:11–20, 1990
33. Arnadottir M, Brattström L, Simonsen O, *et al*: The effect of high-dose pyridoxine and folic acid supplementation on serum lipid plasma homocysteine concentrations in dialysis patients. *Clin Nephrol* 40:236–240, 1993
34. Selhub J, Jacques PF, Wilson PFJ, *et al*: Vitamin status and intake as primary determinants of hyperhomocysteinemia in an elderly population. *JAMA* 270:2693–2698, 1993